Cholinergic Receptors in Cognitive Disorders

E.K. Perry, R.H. Perry, C.J. Smith, D. Purohit, J. Bonham, D.J. Dick, J.M. Candy, J.A. Edwardson and A. Fairbairn

ABSTRACT: Cholinergic receptors (muscarinic subtypes M1 and M2, and putative nicotinic binding) have been examined in the hippocampus obtained at autopsy from a variety of patients with cognitive disorders (Alzheimer's, Parkinson's, and Huntington's diseases, Down's Syndrome and alcoholic dementia) and compared with neurologically normal controls and cases of Motor Neuron disease. In all of the disorders associated with a pre-synaptic cortical cholinergic deficit reflected by an extensive loss of choline acetyltransferase (Alzheimer's disease, Parkinson's disease and Down's Syndrome) there was a substantial reduction in the binding of (3H) nicotine to the nicotinic receptor. By contrast reductions in both muscarinic subtypes (M1 and M2) were apparent to only a moderate extent in Alzheimer's disease, whereas in Parkinson's disease binding was significantly increased (apparently not in relation to anti-cholinergic drug treatment) in the non-demented but not demented cases. A further abnormality detected in Alzheimer's disease but not the other disorders investigated was a decrease in an endogenous inhibitor of nicotinic binding, the identity of which is as yet unknown but which may be a candidate for a possible endogenous modulator of the nicotinic receptor. These observations suggest that in Alzheimer's disease not only muscarinic but also nicotinic receptor function should be considered in relation both to future therapeutic strategies and, in the search for a clinical marker which might be of diagnostic value, to potential probes of the cortical cholinergic system.

RÉSUMÉ: Les récepteurs cholinergiques dans les affections cognitives. Nous avons examiné les récepteurs cholinergiques (muscariniques des sous-types M_1 et M_2 et sites de liaison putatifs nicotiniques) dans des hippocampes provenant de l'autopsie de divers patients souffrant d'affections cognitives (maladies d'Alzheimer, de Parkinson et de Huntington, syndrome de Down et démence alcooloqie) et nous les avons comparés à des témoins normaux au point de vue neurologique et à des patients atteints de maladie du neurone moteur. Dans toutes les affections associées à un déficit cholinergique pré-synaptique au niveau du cortex, déficit réflété par une perte importante de la choline acétyltransférase (maladies d'Alzheimer, de Parkinson et syndrome de Down), il y avait une réduction importante de la liaison de la (3H) nicotine au récepteur nicotinique. A l'opposé, la diminution des deux sous-types muscariniques (M_1 et M_2) était peu importante dans la maladie d'Alzheimer, alors que la liaison était significativement augmentée dans la maladie de Parkinson (sans qu'il y ait de relation apparente au traitement par les anticholinergiques) dans les cas où il n'y avait pas de démence. Nous avons détecté une autre anomalie présente dans la maladie d'Alzheimer, mais absente dans les autres maladies que nous avons investiguées, soit la diminution d'un inhibiteur endogène de la liaison nicotinique, dont l'identité est encore inconnue, mais qui peut s'avérer un candidat possible comme modulateur du récepteur nicotinique. Ces observations nous portent à croire que non seulement la fonction des récepteurs muscariniques, mais aussi celle des récepteurs nicotiniques, devraient être prises en considération dans la maladie d'Alzheimer en relation avec les stratégies de traitement futures et également avec les sondes qui pourraient être utilisées au niveau du système cholinergique cortical dans la recherche d'un marqueur clinique qui posséderait une valeur diagnostique.

Can. J. Neurol. Sci. 1986; 13:521-527

The cholinergic hypothesis[1] continues to provide a useful theoretical basis for investigating the memory disorder of diseases such as Alzheimer's, although in practice the clinician does not yet have any aid to rectifying cholinergic dysfunction. In disorders such as Alzheimer's disease associated with degeneration of cholinergic axons projecting to the cortex, the status of cortical cholinergic receptors is central to the design of potential cholinomimetic therapy. Curiously, reports concerning muscarinic receptors in autopsy tissue vary extensively in Alzheimer's disease and include: normal or reduced classical antagonist binding;[2-4] normal M1 subtype;[5] reduced M2 subtype;[6] reduced M1 and M2 subtypes.[7] Such disparities probably

From the Department of Neuropathology (Drs. E.K. Perry, R.H. Perry, Smith and Purohit); the Department of Pathology (Dr. Bonham); the Regional Neurological Centre (Dr. Dick); the MRC Neuroendocrinology Unit (Drs. Candy and Edwardson) and the St. Nicholas Hospital (Dr. Fairbairn), Newcastle General Hospital, Newcastle Upon Tyne, U.K.

Reprint requests to: Dr. E.K. Perry, Department of Neuropathology, Newcastle General Hospital, Newcastle Upon Tyne, U.K.

reflect a combination of factors, such as variations in methodology or in the severity of cases examined, but, in effect, suggest that extensive changes in muscarinic receptor binding are not (in contrast to reductions in choline acetyltransferase) a major neurochemical feature in Alzheimer's disease. Indeed, in a recent preliminary PET study of (123I) quinuclidinyl benzilate uptake, only a moderate (20%) reduction was noted in the cortex[8] suggesting that in Alzheimer's disease the majority of muscarinic receptors are intact, at least in terms of ligand binding (other aspects of receptor function such as signal transduction and translation are considered in the discussion).

For many years the cerebral nicotinic receptor has been either largely ignored or the subject of occasional inconsistent reports. This reflects the inability of alpha-bungarotoxin (used successfully to probe the neuromuscular receptor) to block the nicotinic response of neuronal cell types[9] despite the presence in brain of toxin binding sites. The situation has at least partly been clarified by the recent demonstration of: (i) A close parallel distribution of non muscarinic (3H) acetylcholine and (3H) nicotine binding in brain, distinct from the pattern of alpha-bungarotoxin binding;[10] and (ii) The isolation of a cDNA clone coding for a neural nicotinic acetylcholine receptor subunit whose identity suggests the alpha-bungarotoxin binding site of the muscle subunit is not conserved in brain[11] — possibly accounting for pharmacological differences between the two receptor types. Based on these observations, either nicotine or acetylcholine appear to be useful probes of the CNS nicotinic receptor. Whilst previous investigations of Alzheimer's disease, employing alpha-bungarotoxin have indicated either normal or decreased binding,[12,13] recent investigations using either nicotine or acetylcholine suggest that nicotinic receptor binding is substantially reduced.[7,14,15]

The present investigation was undertaken to examine both muscarinic and nicotinic receptor binding in the same postmortem brain samples from patients with Alzheimer's disease and Parkinson's disease (in which dementia appears to be associated with cortical cholinergic degeneration) and to compare this with both normal controls and diseases in whom the cortical cholinergic system is apparently intact — Huntington's disease and Motor Neuron disease. Included in the analysis were cases of Down's Syndrome, in which Alzheimer-type pathology is evident with increasing age[16] and cases presenting with alcoholic dementing syndrome in which the cholinergic system may be involved. Results are reported for the hippocampus, an area implicated in the learning process[17] with a functionally important cholinergic input from the septal area,[18] in which as many as 50% of the hippocampal projecting neurons may be cholinergic.[19]

Methods

Cases

Case details are provided in Table 1. Diagnoses were based on standard clinical and neuropathological assessments. With respect to Alzheimer-type pathology (numerous senile plaques and neurofibrillary tangles in most neo- and archicortical areas) this was by definition evident in all cases of Alzheimer's disease and was also apparent in all except one (a 31 y patient) of the cases of Down's Syndrome but was not apparent to any significant extent in the cases of Parkinson's disease — neither the non-demented or demented (differentiated on the basis of a mental test score[20] of above and below 25/37 respectively) in whom no neocortical neurofibrillary tangles were detected. With respect to the cases of alcoholic dementia, although Alzheimer-type pathology was not apparent, two of the cases demonstrated the classical pathological features of Wernicke's encephalopathy. The clinical groups were well matched for autopsy delay although not so well matched for age or gender (Table 1). Thus compared with the control group the majority of the Alzheimer patients were female and the mean ages of the Down's and Motor Neuron groups were younger. With respect to drug treatment, in view of the potential effects of anticholinergic drugs on the cholinergic receptors, this was investigated in detail through retrospective assessment of the case notes in the Parkinson group. Within this group, whilst all except one patient received L-DOPA, 5 (3 non-demented and 2 demented) received anticholinergic medication for at least 1 year before death whereas 6 (4 non-demented and 2 demented) did not. Case details on the Alzheimer patients revealed no evidence of direct anticholinergic treatment in any patient.

Tissue Treatment

Coronal sections from the mid hippocampal formation (300-500 mg) were removed at autopsy, snap frozen and stored in liquid nitrogen. For biochemical assay the tissue was homogenized in 10 vol 10 mM Na/K phosphate buffer, pH 7.4. Aliquots of the homogenate were removed for choline acetyltransferase and acetylcholinesterase enzyme assays as previously described[21] and the remainder centrifuged at 40,000 g for 15 min. at 4°C (with retention of the supernatant for determination of the nicotinic inhibitor) to provide a membrane preparation which was washed twice, stored at −70°C and used for the receptor binding studies.

Receptor Analysis

For muscarinic receptor binding[22,23] membranes were resuspended (3 mg/ml) in phosphate buffer and incubated with 1 nM (3H) N-methylscopolamine (NMS) either alone (total binding),

Table 1

Cases	Number	Age (y, m±sd)	PM delay (h, m±sd)	Gender (m:f)
Normal	11	67±14	34±14	4:7
Alzheimer's disease	8	76±11	39±27	0:8
Down's syndrome	5	50±11	37±12	4:1
Parkinson's disease				
Without dementia	10	73± 7	47±28	5:5
With dementia	4	75± 8	47±22	2:2
Alcoholic dementia	7	60± 6	21±12	5:2
Huntington's disease	4	67± 9	21±15	3:1
Motor Neuron disease	4	54±11	25±18	1:3

https://doi.org/10.1017/S0317167100037240 Published online by Cambridge University Press

with 1 μM atropine (non-specific binding), 0.3 mM carbachol (displacing NMS from the "M2" subtype) or 2 μM pirenzepine (displacing NMS mainly from the M1 subtype). The concentrations of pirenzepine and carbachol were selected on the basis of Kd values for the respective high affinity binding sites derived from displacement curves obtained with normal human hippocampus (Smith et al, in preparation). Following incubation at 25°C for 1h, the labelled membranes were separated by rapid filtration, using a semi-automatic Skatron cell harvester with GF/B filtermats, and washed thrice. Regarding the nomenclature of the muscarinic subtypes it should be noted that whilst designation of the M1 subtype using the non-classical antagonist pirenzepine at a concentration selected to detect mainly the higher affinity binding site is widely accepted, description of high affinity carbachol displaced antagonist binding as "M2" is less well established and although a more accurate term for this binding might be 'high affinity agonist subtype' the term "M2" is employed here for convenience.

For measurement of nicotinic receptor binding[10,24] membranes were resuspended (10 mg/ml) in Tris buffer (50 mM, pH 7.8) and incubated with (−) (3H) nicotine (4 nM) in the presence and absence of 0.1 mM (−) nicotine ditartrate. Following incubation for 20 min. at 25°C membranes were filtered (Millipore manifold) and washed on GF/C filters pre-soaked in 0.1% polylysine. For both receptor analyses filters were counted at least 15h after the addition of Optiphase "MP" (LKB). Receptor binding was expressed in terms of membrane protein.[25]

Nicotinic Inhibitory Factor

Endogenous activity inhibiting the normal binding of (3H) nicotine[26] was estimated in the supernatant fraction from the first centrifugation of the original homogenate (see above) by incubating normal human thalamic membranes (selected for relatively high binding) under conditions identical to those described above, both in the absence and presence of supernatant fraction (at 40 mg/ml — the concentration selected to give under 70% inhibition, a linear relationship having been established between inhibitor concentration and percentage inhibition up to 75% inhibition).

Results

Enzyme Activities

As widely reported previously, both choline acetyltransferase and acetylcholinesterase are significantly decreased in Alzheimer's disease, Down's Syndrome, and Parkinson's disease — more extensively in demented cases of the latter (Table 2). Within the four older (over 50 y) cases in the Down's group, enzyme activities were as low as, and for choline acetyltransferase lower than, those in the Alzheimer group, whereas the enzyme activities were much higher in the younger case (Table 2) although with respect to choline acetyltransferase still approximately half (10.4 n mol/h/mg protein) those in the younger (40 to 50 y) controls (mean of 2 cases: 21.1 n mol/h/mg protein). Amongst the cases of alcoholic dementia choline acetyltransferase activities in the two confirmed cases of Wernicke's encephalopathy (2.4 and 5.6 n mol/h/mg protein) were the lowest of this group and within the Alzheimer range. Enzyme activities in Huntington's and Motor Neuron diseases were within the normal range (Table 2).

Muscarinic Receptors

Compared with the enzymes (above), the muscarinic receptor data (Table 2) were far less variable (standard deviations were generally under 25% of the mean values) and neither total receptor binding nor M1 or M2 subtypes were significantly different in the demented Parkinsonian, Huntington's, Motor Neuron or Alcoholic groups. There was in the non-demented Parkinson group a significant increase in total muscarinic binding which did not reach significance for the subtypes assessed individually (Table 2). In contrast, in Alzheimer's disease there was a significant, although only moderate decrease in both M1 and M2 subtypes (Table 2). Displacement curve analysis indicated normal affinities for the M1 and M2 sites in both the non-demented Parkinson and Alzheimer groups. Within the group of Down's cases although mean receptor binding was normal there was a significant inverse correlation ($r = -0.95$, $p<0.02$ for total binding) with age and in the younger (31 y) case binding was amongst the two highest levels in the entire series (the other being a non-demented case of Parkinson's disease). Within the normal group neither receptor subtype correlated significantly with age (Figure 1) nor did delay or gender influence the data. Regarding drug treatment in the Parkinsonian group, muscarinic receptor binding was not apparently related to anticholinergic drug treatment in either the non-demented or demented sub groups (total muscarinic receptor binding was 258+37 and 256+77 fmol/mg protein in the treated and untreated groups respectively). Within the combined normal and Alzheimer groups but not within any other of the groups consid-

Table 2: Hippocampal cholinergic receptor and enzyme activities (mean ± SD)

Group	Muscarinic Receptor Binding Total	M1 (f mol/mg protein)	M2 (f mol/mg protein)	Nicotinic Binding	Receptor Inhibitor (% at 40 mg per ml)	ChAT nmol/h/mg protein	AChE m U/mg protein
Normal	230±27	180±20	111±11	10.3±4.0	29.1±16.8	14.8±5.2	34.9±11.7
Alzheimer's Disease	194±47	153±40[a]	94±18[b]	5.2±3.4[c]	6.6± 7.8[c]	3.6±2.7[d]	17.2± 6.8[d]
Down's syndrome							
31y	385	290	188	3.9	55.0	10.4	46.2
>50y	217±53	165±39	111±29	4.7±4.0[a]	29.2±21.2	1.6±1.3[d]	19.6± 7.5[b]
Parkinson's Disease							
—without dementia	273±51[a]	213±37	127±29	6.2±3.2[b]	22.9±14.9	8.7±4.6[c]	25.1± 6.5[c]
—with dementia	231±45	182±35	110±24	6.4±2.6	30.6±20.6	2.5±3.1[d]	14.5± 8.8[c]
Alcoholic dementia	236±32	186±26	113±13	10.9±2.2	35.5±10.3	10.5±5.6	26.5± 7.7
Huntington's Disease	220±31	178±30	100±11	8.0±1.6	31.5±10.6	16.3±1.3	34.7± 3.4
Motor neuron Disease	239±23	184±15	107±11	8.8±3.5	54.2±10.8	19.9±3.9	34.7± 3.9

a, b, c, d: significantly different from the normal group (Mann Whitney U Test) $p<0.05$, <0.02, <0.01 and <0.001 respectively.

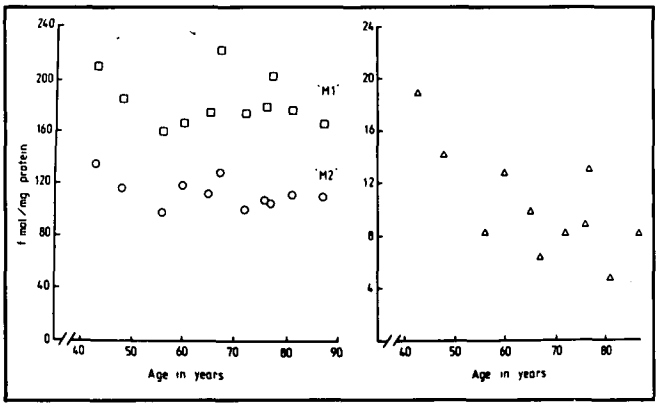

Figure 1 — Muscarinic (LHS) and Nicotinic (RHS) receptor binding as a function of age in normal human individuals. Correlation with age was significant ($p<0.002$) for the nicotinic ($r = -0.69$) but not muscarinic (M1, $r = -0.22$ and M2, $r = -0.46$) receptors.

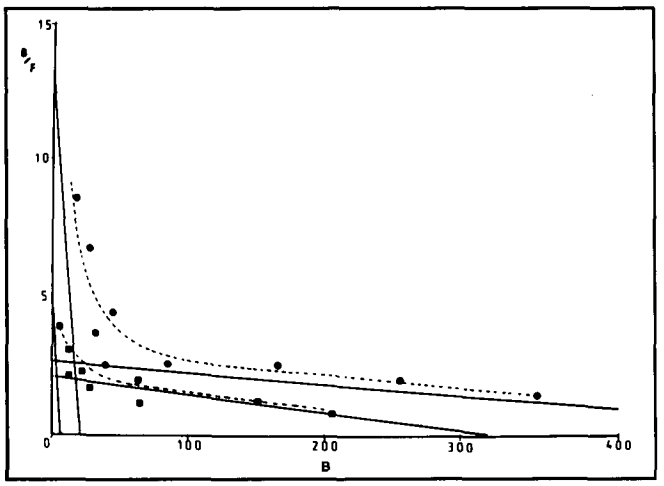

Figure 2 — Scatchard analysis of (H)-nicotinic binding to pooled hippocampal gyral membranes from the normal (●) and Alzheimer (■) group. Computer fitting of the plot to a 2-site model yielded normal values of $Bmax_1 = 19$, $Bmax_2 = 689$ f mol/mg protein and $Kd_1 = 1.5$, $Kd_2 = 280$ nM and values for Alzheimer's disease of $Bmax_1 = 5$, $Bmax_2 = 310$ f mol/mg protein and $kd_1 = 1.0$, $kd_2 = 148$ nM.

ered separately, there was a significant correlation between the decrease in choline acetyltransferase activity and reduction in M2 receptors ($r = 0.68$, $p<0.01$).

Nicotinic Receptor

(3H) nicotine binding was, in contrast to muscarinic binding, strongly correlated with age within the normal group, decreasing substantially between the ages of 40 and 60 y (Figure 1). Compared with the control group binding was significantly reduced in Alzheimer's disease, Parkinson's disease (both with and without dementia) and Down's Syndrome but not in Huntington's disease, 'alcoholic' dementia or Motor Neuron disease (Table 2). The reduction in binding in the Alzheimer cases was related to a decrease in Bmax, kd values — at least for the high affinity site — being unchanged (Figure 2). Unlike the muscarinic receptor in the Down's group or choline acetyltransferase in the alcoholic dementia group, nicotine binding did not fall outside the group range in the younger case of Down's Syndrome or in the cases of Wernicke's encephalopathy within the Alcoholic Dementia group. Nicotine binding was not affected by autopsy delay or gender nor, within the Parkinsonian group, by anticholinergic drug treatment. There was, however, a significant correlation between the reduction in nicotinic receptor and decrease in choline acetyltransferase within the combined normal, Alzheimer and Parkinson groups ($r = 0.49$, $p<0.01$).

Nicotinic Inhibitor

As with the nicotinic receptor, the inhibitory factor correlated strongly with age within the normal group ($r = -0.75$, $p<0.01$) but was unrelated to gender or autopsy delay. Compared with the normal group, matched for age, (Table 1) the inhibitor was significantly and extensively (over 70%) reduced in Alzheimer's disease (Table 2) but not in the other groups investigated. IC 50 values for pooled hippocampal extracts from the normal and Alzheimer groups were, respectively, 31 and 87 mg (original tissue weight)/ml. Within the Down's group the inhibitor was highest (55%) in the youngest case and when the older Down's cases were compared with younger (age matched) controls or Motor Neuron disease there was a trend towards decreased inhibitor. The reduction of the inhibitor in Alzheimer's disease is unlikely to be the result of a nonspecific influence such as autopsy delay, drug treatment or agonal status since these were similar in the other groups (for example Huntington's and Motor Neuron disease), subject to similar variable factors. In the Alzheimer group the inhibitor did not correlate with either the level of the receptor itself nor with the activity of the enzyme choline acetyltransferase.

Discussion

Muscarinic Receptors, the Cholinergic System and Dementia

The muscarinic receptor subtypes M1 and M2 are differentiated physiologically by their relation to changes in potassium ion conductance,[27] a decrease and increase in conductance being associated with respectively M1 and M2 types (the latter also being associated with other effects such as decreased calcium conductance). At the cellular level it has been suggested that whilst M1 receptors are predominantly post-synaptic, M2 subtypes are localised on the pre-synaptic cholinergic nerve terminal where they may modulate acetylcholine release.[6] At the biochemical level, such as in the present investigation, the differentiation between the two subtypes has been achieved using select concentrations of non-classical muscarinic antagonist pirenzepine (M1) and the muscarinic agonist carbacol (M2). That this procedure is at least partially successful in differentiating the two subtypes is suggested by recent findings of different regional variations of the two types in adult human brain (unpublished data) and of developmental changes in the ratio, M1/M2, in the fetal human brain.[28] Nevertheless there is unavoidably a certain degree of overlap between the two subtypes in the present estimations and indeed definitive studies of the M2 subtype await the development of a specific CNS antagonist. A further technical problem relating to the investigation of receptors in human diseases such as Alzheimer's is the degree of tissue atrophy which occurs in the disease process itself. Thus for example the hippocampus is a site of particularly marked atrophy in Alzheimer's disease and it could be postulated that reductions in such components as receptors may occur, but when expressed in terms of unit weight or protein would not be detected because of the decrease in tissue mass. Thus results from studies such as these comparing different diseases may be complicated by the extent of tissue atrophy,

https://doi.org/10.1017/S0317167100037240 Published online by Cambridge University Press

a factor which must also be considered when comparisons are made with the lesioning experiments in laboratory animals. The above considerations may account partially for the results of the present study which indicate neither gross abnormalities in muscarinic binding levels in any of the diseases investigated nor selective changes in M1 or M2 sites.

The moderate decrease in both M1 and M2 subtypes in Alzheimer's disease is at variance with previous reports of selective and more extensive M2 reductions,[6,29] and indeed within the Alzheimer group the present data indicate variations in the status of M2 subtype, ranging from normality to substantial (over 50%) reductions, suggesting M2 loss may occur at later stages in the disease process. The normality of the M2 subtype in both demented Parkinsonian cases and older cases of Down's Syndrome (both associated with extensive pre-synaptic cholinergic abnormalities) suggests that, at least in the hippocampus, a predominantly pre-synaptic localisation of the M2 subtype is unlikely. This conclusion is further supported by two recent investigations in rat brain[30,31] indicating only moderate (15-25%) reductions in both subtypes, following lesions of the nucleus of Meynert in rat brain — with the caveat that Meynert and septal lesions may possibly differ in this respect.

An interesting point in relation to both Down's Syndrome and Parkinson's disease is the indication of increased muscarinic binding in both the younger case of Down's Syndrome (without Alzheimer pathology) and non-demented cases of Parkinson's disease, in which the cholinergic enzyme reductions were less marked compared with the older cases of Down's Syndrome and demented cases of Parkinson's disease, both with normal muscarinic receptor binding (Table 2). Perhaps the onset of dementia is associated with gross degeneration of cholinergic axons, a degeneration no longer compensated by muscarinic receptor supersensitivity. In Parkinson's disease the situation is complicated by the possible effects of anti-cholinergic drug treatment. Although animal experiments indicate that anti-cholinergic drugs increase muscarinic receptor binding the present results in Parkinson's disease suggest that increased receptor binding is not the result of such drug treatment.

One of the most intriguing aspects to emerge from these comparative studies is the varying degree of cholinergic involvement in the different dementias investigated. Thus whilst dementia in Alzheimer's disease, Parkinson's disease and middle aged Down's Syndrome are associated with cholinergic degeneration and variable muscarinic receptor abnormalities, the cognitive impairments of Huntington's disease and dementia associated with alcoholism (other than Wernicke's encephalopathy) do not apparently involve the cholinergic system. The normality of the cortical cholinergic system in Huntington's disease (both in terms of the cholinergic enzyme and receptor) has previously been reported.[32] The condition of the cholinergic system in 'alcoholic' dementia is less clear and our findings on two cases of Wernicke's encephalopathy of low choline acetyltransferase activity but normal muscarinic receptor clearly need to be extended to larger numbers of cases, before conclusions can be drawn. Interestingly there is one report[33] of neuronal loss from the nucleus of Meynert in Korsakoff's psychosis.

In Parkinson's disease an unanswered question is the functional significance of the loss of pre-synaptic cholinergic activity in patients without apparent dementia (with a mental test score of above 25). This finding is open to two possible interpretations: (i) moderate (under 50%) reductions in choline acetyltransferase are not clinically significant; or (ii) a moderate reduction in pre-synaptic cholinergic activity is associated with mild cognitive impairment, noted in many patients with Parkinson's disease in whom gross dementia is not evident. Thus, for example, in one recent study of 67 Parkinsonian patients, memory (information processing and learning) was noted to be inferior to the normal.[34] A key question then is whether cholinergic degeneration to the cortex relates to the mild cognitive impairment evident in Parkinson's disease or to the classical features of dementia such as occur in Alzheimer's disease. The answer to this question is clearly relevant to the prospects of cholinergic therapy in dementia. Thus, if the more severe dementing features of Alzheimer's disease are not related to cholinergic degeneration but rather to some other transmitter involvement, then cholinergic replacement therapy is unlikely to be of great value except perhaps in the earliest stages of the disease.

The neuropsychological and memory deficits evident in Alzheimer's disease, Huntington's disease and Korsakoff's encephalopathy have recently been compared and found to be distinct within the differing disease processes.[35] For example, on recall testing all three groups were equally impaired at the shorter delay although at a longer delay the Huntington's and Korsakoff's patients performed significantly better than the Alzheimer's group. Clearly without further information on the involvement of individual cortical transmitter systems in the different disease processes it would be premature to draw conclusions regarding the role of the cholinergic system in particular cognitive functions. It is, however, tempting to consider that the cholinergic transmitter system plays a central role in cortical plasticity and that the response of the muscarinic receptor, — of slow onset and relatively long duration, whereby intracellular effects may outlast the receptor interaction — may be related to longer term adaptive processes involved in information storage.

An important issue regarding the status of muscarinic receptors is the inability of standard ligand binding studies to monitor the actual functioning of the receptor molecules. Muscarinic receptors are generally considered to be coupled at least partially via a GTP-binding protein, either directly or indirectly (through such mechanisms as the phosphoinositide response) to the functional change in membrane conductance. One possibility in, for example, Alzheimer's disease is that deficits in transduction or translation mechanisms could, despite the normality or near normality of transmitter binding, result in impaired receptor response. Preliminary investigations in parietal cortex (unpublished observations, C. Smith) suggest that changes in agonist displaced NMS binding induced by the non-hydrolysable GTP analogue (5′ guanglylimidodiphosphate) — a measure of the degree of muscarinic receptor coupling to the G binding protein — are in fact preserved in Alzheimer's disease. This suggests that the majority of muscarinic receptors coupled to G proteins may be functionally intact, an observation which is important in relation to the efficacy of muscarinic agonists in Alzheimer's disease.

Nicotinic receptors and the cholinergic system

The present finding of decreased nicotinic binding in the hippocampus is in agreement with the recent report of Whitehouse et al[15] that both acetylcholine and nicotine binding are substantially reduced in the four cortical lobes in Alzheimer's

disease. Contradictory data from the group of Shimohama[14] suggesting nicotine binding is normal in the cortex despite extensive reductions in subcortical areas such as the Meynert nucleus, may well reflect the technical limitations of employing unwashed membrane preparations.[14] The presence of a nicotinic receptor inhibitor in the brain,[7,26] which is apparently more active in cortical compared with subcortical areas such as the Meynert nucleus (unpublished data) suggests unwashed membranes, may be unsuitable for nicotinic receptor investigations particularly in the cerebral cortex.

The loss of nicotine binding not only in Alzheimer's disease but also in Parkinson's disease and Down's syndrome — all involving pre-synaptic cholinergic degeneration — suggests the nicotinic receptor is either directly or indirectly associated with the pre-synaptic cholinergic nerve terminal. That the receptor may be regulated by the pre-synaptic terminal rather than actually situated on it is indicated by the results of cDNA probe studies in animal brain[11] which demonstrate relatively high activity in the hippocampus where it is presumably associated with intrinsic cell bodies. In brain areas such as striatum the nicotinic receptor is situated on dopaminergic nerve terminals.[36] Whether a dopaminergic localisation of nicotinic binding complicates interpretation of the present results in Parkinson's disease depends on whether the hippocampus receives a significant dopaminergic innervation — an as yet unclear issue in the human. Thus, the loss of nicotine binding in the hippocampus in Parkinson's disease might reflect degeneration of the mesocortical dopaminergic system together with degeneration of cholinergic axonal processes derived from the septum. This could explain the lack of distinction between the non-demented and demented Parkinsonian cases in which dopaminergic terminals presumably degenerate to a similar extent. In Alzheimer's disease the nicotinic receptor loss is more likely to be associated with cholinergic degeneration since by most accounts the cortical dopaminergic system is not affected in this disorder.

In contrast to the muscarinic receptor the nicotinic receptor is not coupled to a signal translation system but instead itself comprises the chemically gated ionic channel. Thus, unless the protein molecule is structurally abnormal in the disease (and normal Kd values suggest it is not) reductions in agonist binding are likely to be directly related to reduced physiological responses. However, an unknown factor — relating to the use of membrane preparations — is the extent to which the receptors are clustered at the synaptic junction rather than dispersed throughout pre- or post-synaptic processes. High resolution autoradiographic studies should ultimately settle this issue.

The discovery of reduced nicotinic receptor inhibitory factor raises interesting questions in relation both to the factor itself and to the pathology of Alzheimer's disease. An inhibitory factor in rat brain has previously been noted to reduce the binding of either acetylcholine or nicotine but not a range of other transmitter specific ligands such as muscarinic, serotonergic or noradrenergic.[26] The factor is of low molecular weight and heat stable. One obvious candidate is choline, which is known to act as a weak nicotinic agonist. However, the regional distribution of the nicotinic inhibitor in human brain (E. Perry, unpublished data) suggesting it is, in contrast to choline, relatively concentrated in the neocortex compared with for example striatum, together with the normality of choline[37] but extensive inhibitor reduction (Table 2) in Alzheimer's disease, suggests it is unlikely to be choline itself. In contrast to choline, acetylcholine is so unstable postmortem that the low levels remaining in autopsy human brain tissue would be unlikely to interfere with the nicotinic receptor binding and moreover no such inhibitory effects by brain supernatant fractions have been noted for the muscarinic receptor (unpublished observation). Whilst identification of this factor is currently underway it might be suggested that, analagous with the benzodiazepine receptor (which constitutes a modulatory sub-site of the GABA receptor, that may bind endogenous anxiogenic peptides), the nicotinic acetylcholine receptor may contain modulatory subsites binding molecules other than acetylcholine. The brain may contain its own nicotine-like modulator whose synthesis and interaction with the nicotinic receptor governs the response to acetylcholine. Whatever the nature of this endogenous compound, it is noteworthy that it is decreased in the cortex but not caudate (E. Perry, unpublished observation) in only those dementing disorders associated with Alzheimer-type pathology (including not only Alzheimer's disease but also Down's syndrome — although in the latter instance further studies are required to confirm the inhibitor reductions). Non-human neuronal tissue has also been noted to contain a factor inhibiting alpha-bungarotoxin binding[38] and in this instance it has been proposed that the interaction may be related to trophic functions. One speculation is that the reduction in nicotinic inhibitor in Alzheimer's disease might somehow be related to degenerative processes, intrinsic to the cortex itself, specifically associated with Alzheimer's disease but not the other diseases investigated such as Parkinson's and Huntington's.

In conclusion, the present investigation together with the numerous reports of others continue to raise intriguing questions regarding the neurochemical pathology of the cholinergic system in dementia and hopefully these research paths will soon reach the objective of providing a useful treatment for such disorders as Alzheimer's disease.

Acknowledgements

This study was supported by the Medical Research Council and Astra; Dr. C. Whitford kindly conducted the Scatchard computer analysis and Judith Green typed the manuscript.

References

1. Perry EK. The Cholinergic hypothesis — ten years on. Brit Med Bull 1986; 42: 63-69.
2. Perry EK, Perry RH, Blessed G, Tomlinson BE. Necropsy evidence of central cholinergic deficits in senile dementia. Lancet 1977; 1: 189.
3. Davies P, Verth AH. Regional distribution of muscarinic acetylcholine receptor in normal and Alzheimer's type dementia brains. Brain Res 1978; 138: 385-392.
4. Rinne JO, Rinne JK, Laasko K, Paijarvi L, Rinne UK. Reduction in muscarinic receptor binding in limbic areas of Alzheimer brain. J Neurol Neurosurg Psychiat 1984; 47: 651-653.
5. Cauldfield MP, Straughan DW, Cross AJ, Crow T, Birdsall NJM. Cortical muscarinic receptor subtypes and Alzheimer's disease. Lancet 1982; 2: 1277.
6. Mash DC, Flynn DD, Potter LT. Loss of M2 muscarinic receptors in the cerebral cortex in Alzheimer's disease and experimental cholinergic denervation. Science 1985; 228: 115-117.
7. Candy JM, Johnson M, Perry EK, Perry RH, Smith C. Hippocampal cholinergic muscarinic and putative nicotinic receptors in human cognitive disorders. Brit J Pharmac 1986 (in press).
8. Holman BL, Gibson RE, Hill TC, Eckelman WC, Albert M, Reva RC. Muscarinic acetylcholine receptors in Alzheimer's disease. JAMA 1985; 254: 3063-3066.

9. Oswald RE, Freeman JA. Alpha-bungarotoxin binding and central nervous system nicotinic acetylcholine receptors. Neurosci 1981; 6: 1-14.
10. Clarke PBS, Schwartz RD, Paul SM, Pert CB, Pert A. Nicotinic binding in rat brain; autoradiographic comparison of (3H) acetylcholine, (3H) nicotine and (121I)α-alpha-bungarotoxin. J Neurosci 1985; 5: 1307-1315.
11. Boulter J, Evans K, Goldman D, Martin G, Treco D, Heinemann S, Patrick J. Isolation of a cDNA clone coding for a possible neural nicotinic acetylcholine receptor α-subunit. Nature 1986; 319: 368-374.
12. Davies P, Feisullin S. Postmortem stability of α-bungarotoxin binding sites in mouse and human brain. Brain Res 1981; 216: 449-454.
13. Lang W, Henke H. Cholinergic receptor binding and autoradiography in brains of non-neurological and senile dementia of Alzheimer-type patients. Brain Res 1983; 267: 271-280.
14. Shimohama S, Taniguchi T, Fujiwara M, Kameyama M. Changes in nicotinic and muscarinic cholinergic receptors in Alzheimer type dementia. J Neurochem 1986; 46: 288-293.
15. Whitehouse PJ, Martino AM, Antuono PG, Lowenstein PR, Coyle JT, Price DL, Kellar KJ. Nicotinic acetyllcholine binding sites in Alzheimer disease. Brain Res 1986; 371: 146-151.
16. Wisniewski KE, Dalton AJ, Crapper McLachlan DR, Wen GY, Wisniewski HM. Alzheimer's disease in Down's Syndrome; Clinicopathological studies. Neurol 1985; 35: 957-961.
17. Olton DS. Memory functions and the hippocampus. *In*: Neurobiology of the hippocampus, Seifert W, ed. Academic Press, London 1983; 335-373.
18. Nicoll RA. The septo-hippocampal projection: a model cholinergic pathway. Trends Neurosci 1985; 8: 533-536.
19. Alonso A, Kohler CH. A study of the reciprocal connections between the septum and the entorhinal area using anterograde and retrograde axonal transport methods in the rat brain. Comp Neurol 1984; 225: 327-343.
20. Blessed G, Tomlinson BE, Roth M. The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. Brit J Psychiat 1968; 114: 797-811.
21. Perry EK, Curtis M, Dick DJ, Candy JM, Atack JR, Bloxham CA, Blessed G, Fairbairn A, Tomlinson BE, Perry RH. Cholinergic correlates of cognitive impairment in Parkinson's disease: comparisons with Alzheimer's disease. J Neurol Neurosurg Psychiat 1985; 48: 413-421.
22. Watson M, Yamamura HI, Roeshe WR. A unique regulatory profile and regional distribution of 3H-pirenzepine binding in the rat provide evidence for distinct M1 and M2 muscarinic receptor subtypes. Life Sci 1983; 32: 3001-3011.
23. Birdsall NJM, Burgen ASV, Stockton YH. Muscarinic receptor heterogeneity. Trends Pharmacol Sci 1984; suppl. (Jan.): 4-8.
24. Shimohama S, Taniguchi T, Fujiwara M, Kameyama M. Biochemical characterization of the nicotinic cholinergic receptors in human brain: binding of $(-)-^{3}H$) nicotine. J Neurochem 1985; 45: 604-610.
25. Lowry OH, Rosenbrough NJ, Farr AI, Randall RJ. Protein measurement with the Folin Phenol reagent. J Biol Chem 1951; 193: 265-275.
26. Sershen H, Reith MEA, Hashim A, Lajtha A. Endogenous material in brain inhibiting (3H) nicotine and (3H) acetylcholine binding. J Neurosci Res 1984; 12: 563-570.
27. North RA. Muscarinic receptors and membrane ion conductance. Trends Pharmacol Sci 1986; suppl. (Feb.): 19-22.
28. Perry EK, Smith CJ, Atack JR, Candy JM, Johnson M, Perry RH. Neocortical cholinergic enzyme and receptor activities in the human fetal brain. J Neurochem 1986 (in press).
29. Whitehouse PJ, Kopajtic T, Jones BE, Kuhar MJ, Price DL. An in vitro receptor autoradiographic study of muscarinic cholinergic receptor subtypes in the amygdala and neocortex of patients with Alzheimer's disease. Neurol 1985; 35 (suppl.): 217.
30. De Belloroche J, Gardiner IM, Hamilton MH, Birdsall NJM. Analysis of muscarinic receptor concentration and subtypes following lesion of the rat substantia innominata. Brain Res 1985; 340: 201-209.
31. Watson M, Vickroy JW, Fibiger HC, Roeske WR, Yamamura HI. Effects of bilateral ibotenate — induced lesions of the nucleus basalis magnocellularis upon selective cholinergic biochemical markers in the rat anterior cerebral cortex. Brain Res 1985; 346: 387-891.
32. Yamamura HI, Wastek GJ, Johnson PC, Stern Z. Biochemical characterization of muscarinic cholinergic receptors in Huntington's disease. Adv Behav Biol 1978; 24: 35-47.
33. Arendt T, Bigl V, Arendt A, Tennstedt A. Loss of neurons in the nucleus basalis of Meynert in Alzheimer's disease paralysis agitans and Korsakoff's disease. Acta Neuropath 1983; 61: 101-108.
34. Heitanen M, Teravainen H. Cognitive performance in early Parkinson's disease. Acta Neurol Scand 1986; 73: 151-159.
35. Moss MB, Albert MS, Butter SN, Payne M. Differential patterns of memory loss among patients with Alzheimer's disease, Huntington's disease and alcoholic Korsakoff's Syndrome. Arch Neurol 1986; 43: 239-246.
36. Clarke PBS, Pert A. Autoradiographic evidence of nicotine receptors on nigrostriatal and meso limbic dopaminergic neurons. Brain Res 1985; 348: 355-358.
37. Richter JA, Perry EK, Tomlinson BE. Acetylcholine and choline levels in postmortem brain tissue: preliminary observations in Alzheimer's disease. Life Sci 1980; 26: 1683-1689.
38. Quik M, Fournier S, Trifaro JM. Modulation of nicotinic α-bungarotoxin binding site in chromaffin cells in culture by a factor (s) endogenous to neuronal tissue. Brain Res 1986; 11-20.